

Isolation and preliminary characterization of a new bacteriophage against *Sphaerotilus natans*

R. Ferreira*, R. Amado*, J. Padrão, S. Santos, V. Ferreira, S. Cortez, J. Azeredo, M. Mota, A. Nicolau
CEB – Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal.

*protozoa@deb.uminho.pt



Activated sludge process, the most commonly used system for biological wastewater treatment, is driven by a community of microorganisms that play a key role in the conversion of the organic matter and removal of nutrients from wastewater. In this artificial ecosystem, filamentous and floc-forming bacteria co-exist in a dynamic equilibrium. However, filamentous bacteria overgrowth lead to a decrease of performance of the plant by causing two well-known phenomena: bulking and/or foaming. *Sphaerotilus natans* is a filamentous bacterium that can cause or contribute to the malfunction of these systems by leading to bulking occurrence. Bacteriophages are regarded as possible novel treatment against the filamentous overgrowth. In this work, the isolation and preliminary characterization of a new lytic bacteriophage against *S. natans* are described. Results clearly indicate the potential effect of this bacteriophage for future wastewater treatment system management.

Introduction

Bacteriophages (phages) are viruses that specifically infect bacteria. They are the most abundant entities in the biosphere, being found in every environment where their bacterial hosts are present [1]. Phages are parasites that invade the bacterial cells and reproduce themselves by using the bacterial machinery. These have an easy, rapid and relatively inexpensive production. *S. natans* is a rod-shaped Gram-negative microorganism [2]. This specie is aerobic and growth at 28 °C [3].

In this work, one lytic phage for *S. natans* was isolated, using sewage water from an urban WWTP. The phage was tested against other species found in WWTP to conclude about its lytic spectra. Stability of the phage solution at different temperatures and pH was determined. Morphologic analysis by Transmission Electron Microscopy (TEM) of the *S. natans* phage was also performed.

Materials and Methods

A culture of *S. natans* (DSM 6575) from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) collection was used to isolate the phages. For the host range screening of the isolated phage, strains of *Sphaerotilus montanus*, *Gordonia amarae*, *Rhodococcus rhodochrous*, *Staphylococcus epidermidis* and *Escherichia coli* were used.

Sewage water from a municipal WWTP was used to isolate phages of *S. natans*. Spot assays were performed against bacterial lawn to check the presence of phages. Plaque picking was repeated until single-plaque morphology was observed.

Thermal stability tests were carried out at -20 °C, 4 °C (as control), room temperature (25 °C), 28 °C 40 °C and 50 °C for 24 h. Similarly, the effect of pH was also evaluated using a universal pH buffer with pH adjusted to 3, 4, 5, 6, 7 (as control), 9, 10, 11 and 12 at room temperature for 24 h.

Transmission Electron Microscopy (TEM) was used to morphologically characterize the *S. natans* phage.

Results

New phage against filamentous bacteria found in activated sludge. This work allowed to isolate one *S. natans* phage. Plaque

morphology of the *S. natans* phage was characterized as clear and uniform small plaques on the host strain.

Host range screening. *S. natans* phage did not show a lytic effect against any of the other bacteria tested.

pH and temperature stability. The results demonstrate that *S. natans* phage was stable after 24 h, at 4 °C, 21 °C, 28 °C and 40 °C, showing a concentration about 7 orders-of-magnitude. At -20 °C and 50 °C, *S. natans* phage concentration decreased about 1 and 1.5 orders-of-magnitude, respectively, in comparison with the control (4 °C).

S. natans phage was stable in the pH range 5.0-11.0. In the pH range 3.0, 4.0 and at pH 12.0, the *S. natans* phage was completely inactivated.

TEM of *S. natans* phage. According to the morphological evaluation [4], the isolated phage must be included in the *Caudovirales* order, *S. natans* phage has a short non-contractile tail and an icosahedral head and consequently was considered to belong to the *Podoviridae* family.

Conclusions

One new *S. natans* phage was isolated and its lytic spectrum and stability at different temperatures and pH values determined. The morphologic analyse by TEM was also carried on. The *S. natans* phage shows clear and uniform small plaques on the host strain. *S. natans* phage was stable after 24 h, at 4°C, 21°C, 28°C and 40°C, and in the pH range 5.0-11.0. The TEM analysis indicates that *S. natans* phage belongs to the *Podoviridae* family. From the results obtained it can be hypothesized that this phage has a potential effect on the removal of the excess of filamentous bacteria and might be used for future wastewater treatment system management.

Further work is required to determine if *S. natans* phage can control *S. natans* overgrowth phenomena in wastewater treatment plants, by using lab-scale reactors.

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